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Original Article

Sterility Maintenance Assessment of Moist/Wet Material After Steam Sterilization and 30-day Storage

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Moist/wet materials stored after autoclaving are considered contaminated and not recommended for use. This study evaluates the maintenance of sterility in moist/wet material after being submitted to steam sterilization and stored for a period of 30 days. Aiming to support decision-making in emergency situations, 40 surgical boxes packed in nonwoven cloth covering Spunbound, Metblouwn, Spunbound (SMS): half (the experimental group) were placed in an autoclave but the drying phase was interrupted, yielding moist/wet materials and the other half (the negative control group) underwent the complete cycle. The external parts of each surgical box were deliberately contaminated with Serratia marcescens and subsequently stored for 30 days. After this period, the boxes' contents were submitted to sterility tests and no growth was observed. The presence of moisture inside the surgical boxes did not interfere with maintaining their sterility.

Descriptors: Sterilization; Surgical Instruments; Contamination; Cross Infection.

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Avaliação da manutenção da esterilidade de materiais úmidos/molhados após a esterilização por vapor e armazenamento por 30 dias

Consideram-se contaminados os artigos molhados/úmidos, armazenados após autoclavação, não sendo recomendados para uso. O objetivo do estudo foi avaliar a manutenção da esterilidade dos materiais molhados/úmidos, após terem sido submetidos ao processo de esterilização pelo vapor e armazenados por intervalo de 30 dias. Com a finalidade de auxiliar a tomada de decisão em situações emergenciais, foram preparadas 40 caixas cirúrgicas, embaladas em SMS, sendo a metade (experimental) submetida a autoclavação, com fase de secagem interrompida, liberando material molhado/úmido e outras 20 (controle) ao ciclo completo. As partes externas de cada caixa foram propositalmente contaminadas com Serratia marcescens e, posteriormente, armazenadas por 30 dias. Após esse período, os conteúdos das caixas foram submetidos a testes de esterilidade, acusando ausência total de crescimento. A presença de umidade dentro das caixas não interferiu na manutenção da esterilidade do seu conteúdo.

Descritores: Esterilização; Instrumentos Cirúrgicos; Contaminação; Infecção Hospitalar.

Evaluación de la manutención de la esterilidad de materiales húmedos/mojados después de esterilizados con vapor y almacenados por 30 días

Se consideran como contaminados los objetos mojados/húmedos almacenados después de ser esterilizados en autoclave, no siendo recomendados para uso. El objetivo del estudio fue evaluar la manutención de la esterilidad de los materiales mojados/húmedos después de sometidos al proceso de esterilización por vapor y almacenados por un intervalo de 30 días. Con la finalidad de auxiliar en la toma de decisiones en situaciones de emergencia, fueron preparadas 40 cajas quirúrgicas embaladas en SMS, siendo la mitad (experimental) sometidas a un proceso de esterilización en autoclave, con fase de secado interrumpida, liberando material mojado/húmedo y otras 20 (control) con el ciclo completo. Las partes externas de cada caja fueron intencionalmente contaminadas con Serratia marcescens y posteriormente almacenadas por 30 días. Después ese período, los contenidos de las cajas fueron sometidos a pruebas de esterilidad, acusando ausencia total de crecimiento. La presencia de humedad dentro de las cajas no interfirió en la manutención de la esterilidad de su contenido.

Descriptores: Esterilización; Instrumentos Quirúrgicos; Contaminación; Infección Hospitalaria.

Introduction

Guaranteeing that critical material used in health care is sterilized and that its sterility is maintained is essential for the quality of care delivered to patients, especially in surgical treatment[1-6]. In practice, even when all standards and recommendations are followed during the sterilization process, material in moist packages or internal content with water droplets might be found. We stress that when the material is packed inside opaque containers, finding that the material is moist/wet is only possible at the point of its use.

Despite recommendations to comply with the standard procedure to re-sterilize moist/wet material, in practice there are some situations that are difficult to manage. For example, when the patient is already anesthetized in the operating room and there is no safe material to serve as a replacement or there is no possibility to wait for it to be re-sterilized.

Despite the fact that the reviewed scientific literature[7-11] addresses various aspects related to moist/wet packages, it leaves an open question: is the phenomenon of capillarity (moisture passing through the package) capable of carrying microorganisms present on packaging 30 days after storage?

In the face of this question, we developed this study to evaluate how well the sterility of moist/wet material is maintained after it is sterilized and stored for a period of 30 days. For this study's hypothesis we sought to confirm that sterility is maintained 30 days after moist/wet surgical instrument boxes are stored, even with the presence of test microorganisms on the boxes' external surface, testing that the packaging bacterial barrier would not allow contamination.

There are several studies addressing the processing of surgical instruments[12-13]. However, none of these articles address the problem of moist/wet packages after autoclaving and storage. Hence, this study provides theoretical support for decision-making regarding moist/wet material after heat sterilization, an issue not addressed in articles published in this or other periodicals.

Method

This is an experimental study, randomized and carried out in the laboratory, where conditions of care practice were taken into account. The independent variables considered in this study were moist/wet material and duration of storage; and the dependent variable was the result of the microbiological cultures.

In Brazil, most health facilities use surgical stainless steel boxes with holes in the sides and bottom with no lids when submitting surgical instruments to steam sterilization, to ensure steam penetrates into the boxes and then will dry. These boxes are wrapped with a proven microbial barrier to allow appropriate transportation and storage. Therefore, 40 surgical boxes of varied sizes were prepared for this study. Current professional practice was reproduced and surgical instruments of several varieties were placed inside the boxes, occupying 80% of each box's capacity. Twenty of these boxes were randomly chosen as the experimental group and submitted to steam sterilization the cycle of which was interrupted in the early drying stage. The other 20 boxes composed the control group and were submitted to the complete cycle of sterilization (pre-vacuum, exposure-sterilization, and drying).

The boxes were sterilized in a pre-vacuum autoclave validated according to the ISO 11134:1994[14] requirements, at a temperature of 134ºC for four minutes. The mechanical parameters achieved in the sterilization cycles were monitored through the equipment records. Biological monitoring was performed through an indicator using *Geobacillus stearothermophillus* 10^6 U.F.C./mL (3M®) and chemical monitoring through a class 6 emulator (Brownie®), inside each box.

The methodology of the Association of Official Analytical Chemists – AOAC[15] recommends the use of carriers with porcelain cylinders or number 2 silk thread for surgical sutures with 6cm in length forming two loops for sterility tests. Sets of porcelain cylinders were prepared for this study and served as test material to be inoculated in a culture medium. Each set of carriers in ring form was composed of four porcelain cylinders (height 7mm, internal diameter 3mm, and external diameter 7mm) jointed by 6cm of surgical suture thread (silk nº2), totaling five carriers to be inoculated in each culture medium[16]. Eight sets of rings of porcelain cylinders were put in each box in the following positions: three on the superior position, two in the intermediate position and three in the inferior position, totaling 160 samples of test material in experimental group and 160 in the control group.

This sample size was computed with the help of a bio-statistics professional considering an expected proportion of 50%, at 5% level of significance and sample power of 99.9%. There is a larger amount of test material in higher and lower positions because these are more vulnerable to contamination: the highest part is closest to the wrap and the external environment and the lowest position, in addition to having the same risk factors, is in contact with water droplets.

Surgical perforated boxes were packed in a layer of nonwoven cloth covering - SMS (*Spunbound, Metblouwn, Spunbound*), KC 300 (Kimberly Clark®), a covering compatible with the process of steam sterilization following AORN 2004[17] and also AAMI 2002[11] recommendations and is used by many health care facilities in Brazil. Despite the manufacturer's recommendation to use two layers, some Brazilian facilities adopt the practice of using only one layer due to a lack of financial resources. Because we aimed to reproduce the practice usually adopted as closes as possible, one layer was used in this study.

All boxes were weighed before and after sterilization in order to detect the presence or absence of moisture (water), considering that the presence of moisture would yield a greater instrument box weight after sterilization.

Afterwards, both the boxes from the experimental group (whose drying phase was interrupted in the sterilization cycle) and the negative control group boxes (submitted to the complete sterilization cycle) were deliberately contaminated on their external faces using gloved hands that were dipped in a *Serratia marcescens* ATCC 14756 10^6 U.F.C./mL culture and dried for 3 minutes in environmental conditions. The contaminated and gloved hands touched the superior, inferior and lateral faces of the boxes. Then, the boxes were stored on distinct perforated shelves in the same environment for 30 days: one shelf for the boxes of the experimental group and another for the control group boxes. The long interval of 30 days was deliberately chosen to constitute a challenge.

To be sure that the *Serratia marcescens* ATCC 14756 test microorganism would survive for 30 days on the package surface, positive control was performed. For that, 20 samples of the SMS covering were also contaminated with the same *Serratia marcescens* culture and were stored inside dried, and later sealed, sterile test tubes on the same perforated shelves where the surgical boxes were stored. The air temperature and relative humidity of the storage environment were controlled by thermo-hydrometer (Minipa®) and varied respectively between 16.5ºC to 25ºC and 51% to 100%.

After a 30-day interval, the boxes were opened using an aseptic technique and each ring of carriers were inoculated in 20mL of soybean-casein culture medium and incubated for 14[18] days in an oven regulated at 22.5ºC. When *Serratia marcescens* is exposed to a temperature above 30ºC it loses its ability to produce the characteristic red pigmentation, which would hinder its identification[19]. Based on these data, we opted to use the oven at a temperature regulated at 22.5ºC, which is considered optimum for growing *Serratia marcescens*[20].

Data analysis consisted of quantitative descriptive analysis through the reading of sterility tests considering positive or negative growth according to turbidity presented by the tubes in a soybean-casein medium containing the test material. Person's Chi-square would be used to compare the proportion of the experimental and control groups[21].

Results

Table 1 presents the comparison of weights before and after steam sterilization of the boxes of the negative control and experimental groups with the respective values of difference (before and after). The experimental group's final weight (after sterilization) was heavier than the initial weight (before sterilization), average difference -0.14%, which evidenced there was moisture in the interior of the boxes of this group and therefore, an absence of moisture in the control group; average difference +0.77%.

Table 1 – Comparison of the weight of instrument boxes, in the control and experimental groups, before and after the autoclaving process. São Paulo, Brazil 2005

Box	Control group weight			Experimental group weight		
	Before (kg)	After (kg)	Difference (%)	Before (kg)	After (kg)	Difference (%)
01	0.684	0.684	0.00	0.922	0.926	0.43
02	0.920	0.918	-0.22	0.828	0.832	0.48
03	0.794	0.790	-0.50	1.252	1.26	0.64
04	0.832	0.832	0.00	1.614	1.628	0.87
05	0.996	0.994	-0.20	1.020	1.030	0.98
06	1.200	1.198	-0.17	1.234	1.240	0.49
07	0.676	0.676	0.00	1.110	1.116	0.54
08	0.714	0.712	-0.28	0.982	0.990	0.81
09	0.978	0.978	0.00	0.778	0.784	0.77
10	1.616	1.614	-0.12	0.794	0.798	0.50
11	0.878	0.878	0.00	5.058	5.132	1.46
12	1.034	1.032	-0.19	4.874	4.908	0.70
13	0.900	0.898	-0.22	3.850	3.872	0.57
14	4.592	4.586	-0.13	2.652	2.676	0.90
15	5.382	5.372	-0.19	4.860	4.912	1.07
16	2.720	2.719	-0.04	5.008	5.046	0.76
17	2.058	2.056	-0.10	4.440	4.470	0.68
18	2.834	2.830	-0.14	4.200	4.248	1.14
19	2.864	2.860	-0.14	3.744	3.780	0.96
20	3.338	3.332	-0.18	2.428	2.464	1.48
Average	1.849	1.846	-0.14	2.452	2.473	0.77

Table 2 presents the microbiological results of cultures of the test material of the experimental and control groups after 30 days of storage.

Table 2 – Distribution of the results of microbiological cultures according to the localization of the rings of porcelain cylinders in the experimental and control groups 30 days after storage. São Paulo, Brazil 2005

Local of rings in the boxes	Experimental	Control
	Result after 30 days of storage	Result after 30 days storage
Inferior	–/60*	–/60*
Intermediate	–/40*	–/40*
Superior	–/60*	–/60*
Total	–/160*	–/160*

Total number of positive growth/total number of samples

Microbiological growth was observed in 100% of the positive control group.

Discussion

The recommendation of several authors[7-11] not to use material that becomes moistened after steam sterilization is grounded on the premise that microorganisms proliferate in moist environments, though there is no evidence for such a statement.

The results presented on Table 2 show microorganisms did not proliferate after 30 days of storage even with the presence of moisture. This result may be due to the fact that the residual water was also sterilized and boxes were wrapped and stored in appropriate conditions. In this context, the effectiveness of the packages' microbiological barrier assumes a key and defining role in the maintaining the sterility of packaged content[22]. In this study, the covering used (SMS KC 300) was an effective biological barrier for material stored for 30 days even after having contact with intense external contamination via test microorganisms. We add that there is consensus concerning the immediate use of wet material sterilized in a flash cycle of steam sterilization because it is believed that water is free of microorganisms as is the material.

Another reason for authors[7-11] to condemn the use of moist/wet material might be related to the capillarity phenomenon, that is, the ability of moisture to pass through the covering and thereby carry microorganisms with it. It is known that bacteria and fungi grow in humid or moist environments with ambient temperatures. Fungi were not used in the experiments because their cells are much larger than bacteria[23]. The smallest size of a known virus is 500 times larger than that of a water molecule[24]. Based on this theoretical framework, passage of water through critical material does not necessarily entail the passage of microorganisms.

The results of this experiment confirmed the study's initial hypothesis. During the 30 days of storage of moist/wet material with test microorganisms present the entire time on the external surface of the surgical boxes, the test microorganisms did not breach the covering or contaminate the interior of the boxes.

A third explanation that might have grounded the authors' position that moist/wet material might be contaminated during storage refers to the occurrence of micro holes in moist/wet coverings; these would become more fragile and the biological barrier would break. This was not evidenced in this experiment, though it is a plausible possibility.

Person's Chi-square test was initially intended to compare proportions of the experimental and negative control groups, however, since no growth was observed, there was no need to compare proportions through statistical inferences.

We stress that studies addressing this same phenomenon were not found, thereby comparison of results was not possible.

Conclusion

The presence of moisture in the interior of perforated surgical boxes submitted to steam sterilization and then wrapped in a SMS sheet did not interfere in the maintenance of its content-sterility even after 30 days of storage.

Given the study's design with appropriate sample size (power of 99.9%), the findings of this study can support decision-making in practice whether to use moist/wet material without harming the delivery of qualified and ethical care.

This study does not intend to contradict standard recommendations that material must be dry after completing the process of steam sterilization, however, it presents scientific evidence to support decision-making in an emergency situation, such as when patients are already anesthetized in the surgical room and professionals only discover wet material at the time of use and when there is no dry material readily available to replace the wet material.

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